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Physiological responses of hybrid striped bass under sedation by several anesthetics

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Abstract

Several aquatic anesthetics, in doses low enough not to change the swimming behavior, alone and in combination with NaCl, were evaluated for their effectiveness to suppress the stress response in sunshine bass (*Morone chrysops* × *Morone saxatilis*). Clove oil, Aqui-S®, metomidate, MS-222, quinaldine, and quinaldine sulfate were used alone or combined with 5 g/l NaCl. The stress response was evaluated by measuring plasma cortisol, glucose and chloride concentrations in fish after exposure to each of the compounds and after exposure in fish subjected to stress while exposed to each of the compounds. Stress was induced by lowering the water in the aquarium for 15 min. Exposure to all of the compounds, except metomidate, resulted in a significant increase in plasma cortisol and glucose. The cortisol increase induced by exposure to NaCl alone was transient, but NaCl did not reduce the response due to stress. The same pattern of response occurred when the anesthetics were given with NaCl. Only exposure to quinaldine resulted in an increased glucose concentration and plasma glucose was higher than controls in stressed fish exposed to clove oil and quinaldine sulfate. Metomidate may have promise in suppressing the stress response in hybrid striped bass; however, the only approved anesthetic for use in food fish is MS-222. Exposure of fish to MS-222 induced the stress response and did not suppress the magnitude of the stress response in fish exposed to low-water stress. These data point out the importance of continuing to develop and approve compounds for use in aquaculture.
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Keywords: Physiological response; Hybrid striped bass; Sedation

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1. Introduction

Sunshine bass are produced by crossing a white bass female (*Morone chrysops*) with a striped bass male (*Morone saxatilis*), and have become the most popular hybrid bass used in aquaculture. During the production and commercial culture of sunshine bass, the fish are seined, graded and transported several times before they reach market. Each of these handling events is difficult for the fish and may cause stress or mortality. Sublethal stress to the fish can suppress the immune system and result in making the fish more susceptible to pathogens. Physiological stress is a non-specific response composed of a primary component considered to be a neuroendocrine hormonal phase that is characterized by sympathetic activation and the secretion of cortisol and epinephrine (Donaldson, 1981). A secondary phase is characterized by an increase in plasma glucose and osmoregulatory disturbances. Cortisol is a steroid hormone with many biological activities, including gluconeogenesis (Freeman and Idler, 1973) and immunosupression (Schreck, 1996). The increase in glucose results from glycogenolysis from liver glycogen and reduces carbohydrate energy stores. A compound that would reduce the severity of the physiological stress responses, particularly cortisol and glucose, would be a benefit to those culturing hybrid striped bass. A variety of water treatments, including salt, buffers and anesthetics, have been used in fish biology to make fish easier to handle during routine procedures such as bleeding and collection of eggs and milt for reproduction. Presently, only sodium chloride and tricaine methanesulfonate (MS-222) are approved by the United States Food and Drug Administration (FDA) for use on food fish in the United States. Aqui-S® (AQUI-S New Zealand, Lower Hutt, New Zealand), as a ''food safe'' anesthetic for use on aquatic species during transportation and harvesting, is registered in New Zealand and Australia and is under review by the FDA (Davidson et al., 2000). If any compounds are to be useful for handling and transport, a concentration that induces sedation, but not full anesthesia, is desirable. Fish transported under full anesthesia can be damaged by contact with the container. A compound that would induce a physiological state where the fish can maintain normal posture and active opercular movement, but is less excitable, and that would reduce the magnitude and duration of the physiological response, should be of great use in aquaculture. The efficacy of a number of aquatic anesthetics was evaluated in low concentrations to reduce physiological indicators of stress in sunshine bass exposed to the compounds and to a confinement stressor while exposed to the compounds.

2. Materials and methods

Selected aquatic anesthetics were screened to identify concentrations that produced mild sedation in hybrid striped bass. Each compound was introduced into 60-l aquaria with six fish; the water was turned off and the fish were observed for 15 min. A low-water stress exposure was achieved by lowering the water volume to 5 l for 15 min and then restoring the original water volume. The water level was selected so that the fish were submersed but were unable to maintain their posture in the tank. The reduction of the water volume was accomplished in 5 min and refilling of the aquarium required 40 min.

2.1. Concentrations of compounds used

Concentrations selected for physiological experiments were the highest dose of each compound that resulted in no marked behavioral change during exposure and the stress protocol. The concentrations selected are those producing Stage 1 anesthesia as described by Schoettger and Julin (1967) and referred to as sedation by Small (2003). At least three concentrations of each compound were tested. Compounds used were MS-222 (Argent Chemical Laboratories, Redmond, WA), clove oil (Sigma, St. Louis, MO), Aqui-S® (AQUI-S), quinaldine (Eastman Kodak, Rochester, NY), quinaldine sulfate (Spectrum Chemical, Gardena, CA), metomidate hydrochloride (Janssen Pharmaceutica, Belgium, purchased from Shamrock Veterinary Clinic, Cross City, FL) and NaCl. The concentrations used and the range of each compound tested are shown in Table 1.

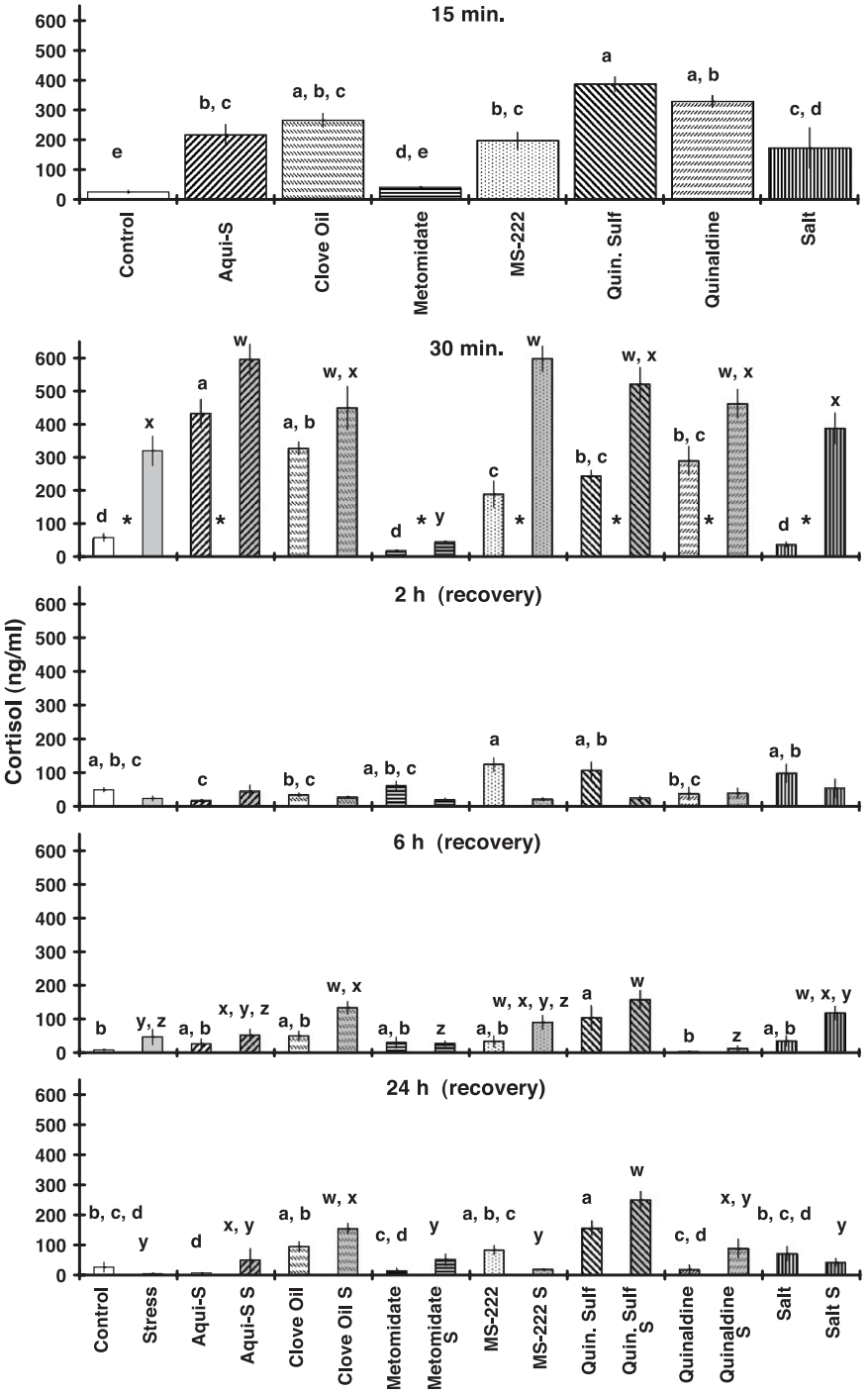
2.2. Exposure protocol

Six hybrid striped bass, 87.0 ± 24.4 g (mean ± S.D., $n = 123$), were stocked into 60-l aquaria and allowed to acclimate in water held at 23 °C. The fish were acclimated for at least 5 days during which they were fed a maintenance diet for both nutrition and to insure they had acclimated to the tanks. Specific treatments were randomly assigned to all aquaria treatments. For each test compound, 10 tanks were used. Six fish from one tank were sampled immediately and the compound was added to the other nine tanks and incoming water turned off. Responses due to the compound alone were determined on individual tanks of six fish each sampled after 15 and 30 min, and the water turned on. Individual tanks of six fish each were sampled 2, 6 and 24 h after restoring the water flow. Responses due to a combination of drug exposure and stress were determined on the remaining four tanks by exposing fish to the test compounds for 15 min and then to the low-water stress described above for the second 15-min. Fish were sampled at the end of the 15 min stress (30 min total exposure, 15 min to compound, plus 15 min to compound and low-water stress), and the water level restored. The exposed/stressed groups were sampled 2, 6 and 24 h after beginning to refill the aquaria to determine recovery. A control group of fish was sampled at each of the same time intervals but without exposure to any compound or to the low-water stress.

Table 1
Compounds to which hybrid striped bass were exposed

Compound	Concentration used	Range of concentrations screened
MS222	25 mg/l	25–75 mg/l
Clove oil	8.0 μl/l	2.5–50 μl/l
Aqui-S®	3.6 mg/l	2.2–5.4 mg/l
Quinaldine	5.0 μl/l	2.5–10 μl/l
Quinaldine sulfate	8.3 mg/l	2.5–10 mg/l
Metomidate	1.5 mg/l	0.05–2.0 mg/l
Sodium chloride	5 g/l	

Concentrations of anesthetics were chosen as the highest level that resulted in no behavioral change during a 15-min exposure followed by a 15-min low-water stress.


15 min.
30 min.
2 h (recovery)
6 h (recovery)
24 h (recovery)
Cortisol (ng/ml)
Control
Stress
Aqui-S
Aqui-S S
Clove Oil
Clove Oil S
Metomidate
Metomidate S
MS-222
MS-222 S
Quin. Sulf
Quin. Sulf S
Quinaldine
Quinaldine S
Salt
Salt S

A second series of tests with 10 tanks was run using each anesthetic in combination with 5 g/l of NaCl. This concentration was chosen because it is an often used concentration in transporting striped bass and hybrid striped bass. The experimental design was similar to the previous experiments. Both NaCl and anesthetic were added at the beginning and groups of six fish were sampled at the same time intervals as before. One set of five tanks was exposed to only the compounds and another set of four tanks was exposed to a 15-min low-water stress in the presence of the compounds.

2.3. Analytical procedures

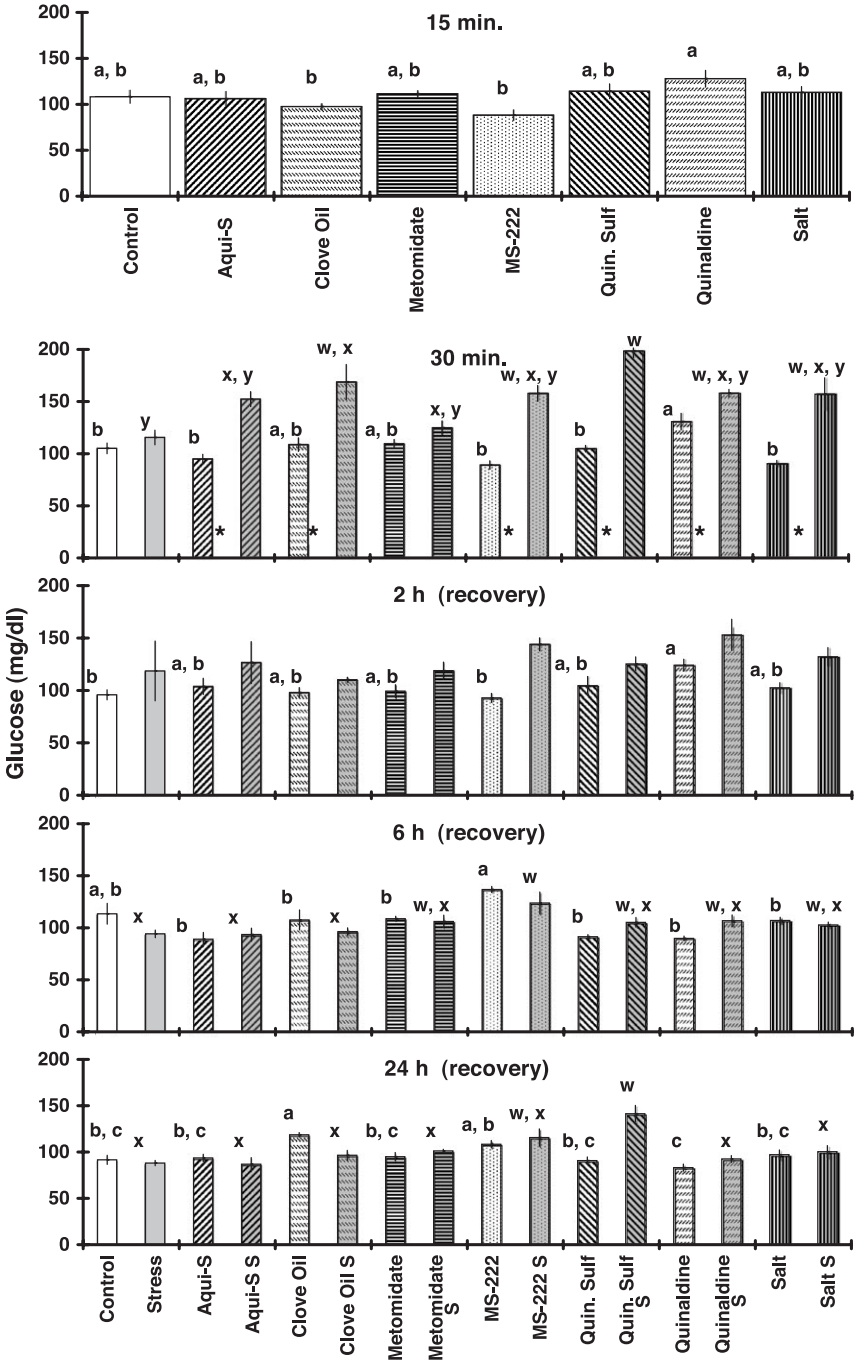
At each time interval, a blood sample was taken from six fish with a heparinized syringe, the blood centrifuged, and the plasma stored frozen. Plasma cortisol concentrations were determined by radioimmunoassay (RIA) using the BioChem ImmunoSystems Cortisol Bridge kit (#14394, Polymedco, Cortlandt Manor, NY) and reported as ng/ml. This kit has not been previously validated for hybrid striped bass. Recovery estimates were performed with hybrid striped bass plasma from unstressed fish spiked with five replicates of 5.0, 50 and 150 ng/ml cortisol standards added to one-half of the volume of plasma from unstressed fish. Concentrations of cortisol measured in the unstressed plasma and the spiked samples were 17.6, 16.9, 62.3 and 147.3 ng/ml, respectively. Dilution experiments were done with 1:1 and 1:3 (plasma:0 standard) of plasma from stressed hybrid striped bass. Concentrations of cortisol were 70.5, 38.2, and 19.5 ng/ml in undiluted, diluted 1:1 and diluted 1:3 plasma, respectively. Within-assay variation was determined on eight replicates of a plasma pool from unstressed fish and fish held in a net for 30 min. The mean and standard deviation of the cortisol concentration of the unstressed fish was 17.6 ± 0.52, and 70.5 ± 3.2 ng/ml from the stressed fish with a coefficient of variation of 2.9% and 4.5%, respectively.

Plasma glucose concentrations were determined by the glucose oxidase procedure (Sigma Diagnostics, No. 510A) and reported as mg/100 ml plasma. The coefficient of variation for analyses over 11 consecutive days of a normal human serum pool was 3.2% as reported by the manufacturer. Ionic plasma chloride was measured with a Corning 925 chloride analyzer and reported as meq/l.

2.4. Statistical analysis

Comparisons among treatments were done by analysis of variance followed by Tukey's multiple range test when statistical significance ($P \leq 0.05$) was indicated. Comparison between stressed and non-stressed groups were done by Student's *t*-test.

Fig. 1. Plasma cortisol concentrations of sunshine hybrid striped bass exposed to low concentrations of several anesthetics for 30 min (open bars) or for 15 min and then exposed to a low-water stress (S after label, shaded bars) followed by a recovery period of 24 h. Letters from the first part of the alphabet indicate statistically similar subsets among fish exposed to the anesthetics only and letters from the last part of the alphabet indicate statistically similar subsets among fish stressed for the last 15 min of the anesthetic exposure by Tukey's multiple range test ($P < 0.05$). Statistical differences between exposed fish and fish exposed and stressed for each treatment by Student's *t*-test ($P < 0.05$) are indicated by an *. Data represent the mean ± S.E.M. for six fish.


15 min.
30 min.
2 h (recovery)
6 h (recovery)
24 h (recovery)
Glucose (mg/dl)
Control
Stress
Aqui-S
Aqui-S S
Clove Oil
Clove Oil S
Metomidate
Metomidate S
MS-222
MS-222 S
Quin. Sulf
Quin. Sulf S
Quinaldine
Quinaldine S
Salt
Salt S

3. Results

Concentrations of plasma cortisol, glucose or chloride did not change during the course of the experiment in control fish that did not receive any treatment or stress (Figs. 1–3). These data are shown as means among time of sampling (vertically).

3.1. Exposure to water treatments

All statistically different subgroups are shown on the figures; however, for clarity, only treatments differing from the controls are discussed here. Plasma cortisol concentrations increased significantly above those of control fish after 15 min in all treatments except the fish exposed to metomidate (Fig. 1). After 30 min of exposure, all treatments except metomidate and NaCl had plasma cortisol concentrations higher than control fish. After 2 h, fish from all treatment groups had cortisol levels similar to control fish. Plasma cortisol concentrations remained similar to controls except for the fish treated with quinaldine sulfate which had a secondary increase above control fish at 6 and 24 h.

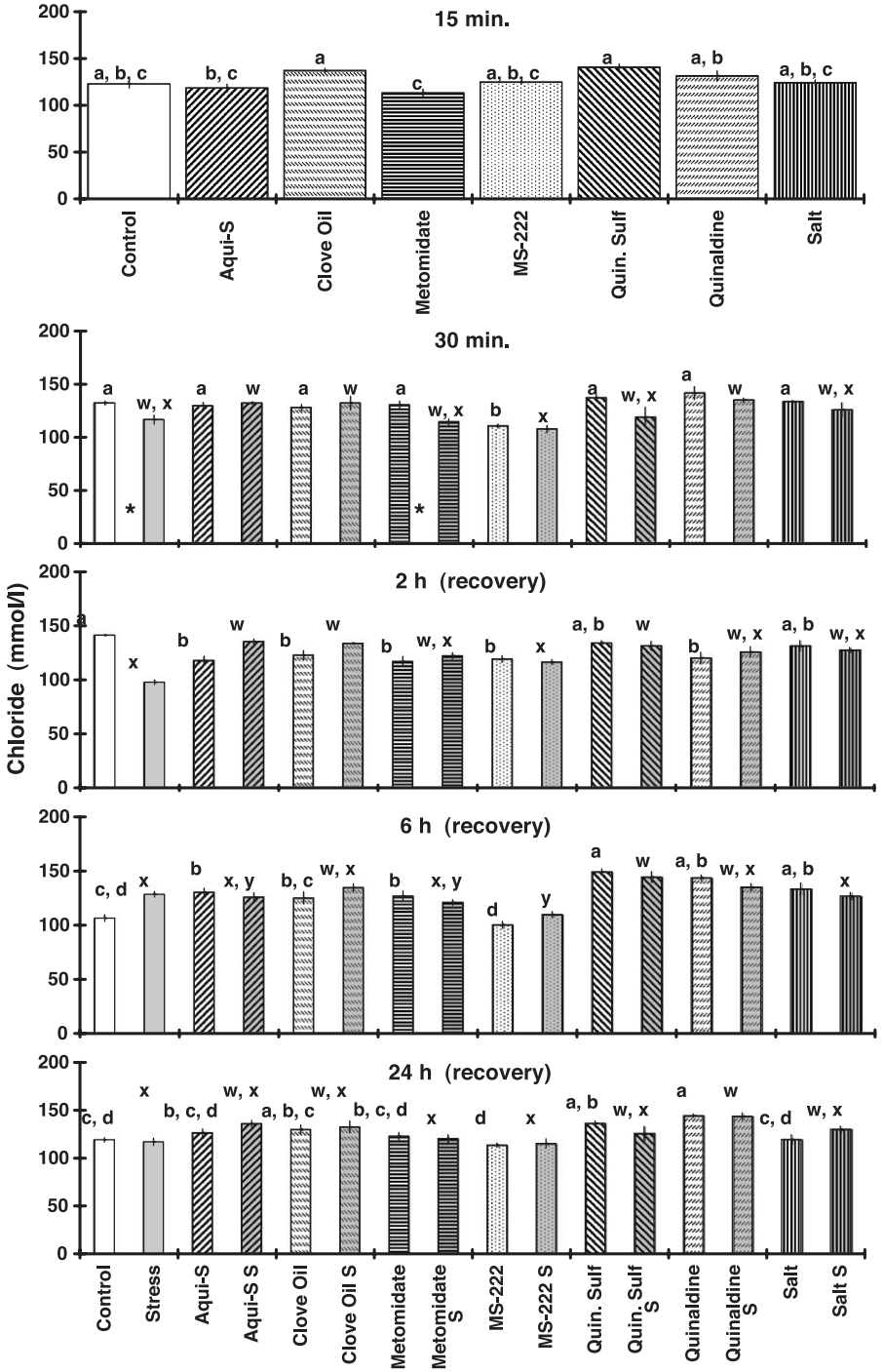
Plasma glucose was fairly stable and no treatment group was different from control levels after 15 min. The same pattern was apparent after 30 min; however, the quinaldine-treated fish had concentrations significantly higher than control fish (Fig. 2). After 2 h of recovery, only quinaldine-treated fish had glucose concentrations different from control. After 6 h of recovery, no treatment was different from controls. After 24 h, clove oil-treated fish had glucose concentrations higher than control fish. Differences during all recovery times were quantitatively small and considered not physiologically meaningful.

Plasma chloride changes in treated fish were small and no group was different from controls after 15 min (Fig. 3). After 30 min, plasma chloride levels in fish treated with MS-222 were lower than control levels. After 2 h of recovery, fish treated with Aqui-S®, clove oil, metomidate, MS-222 and quinaldine were all significantly lower than controls. After 6 h of recovery Aqui-S®-, metomidate-, quinaldine sulfate-, quinaldine- and NaCl-treated fish were higher than controls, and after 24 h of recovery, quinaldine sulfate- and quinaldine-treated fish continued to have chloride concentrations higher than controls.

3.2. Effects of stress during exposure to anesthetics

Fish exposed to low-water stress for 15 min after receiving the test compounds (30 min) had cortisol concentrations higher than non-stressed fish in all treatment groups except clove oil (Fig. 1). Fish stressed while exposed to MS-222 and Aqui-S® had significantly higher cortisol concentrations than stressed fish exposed to the other

Fig. 2. Plasma glucose concentrations of sunshine hybrid striped bass exposed to low concentrations of several anesthetics for 30 min (open bars) or for 15 min and then exposed to a low-water stress (S after label, shaded bars) followed by a recovery period of 24 h. Letters from the first part of the alphabet indicate statistically similar subsets among fish exposed to the anesthetics only and letters from the last part of the alphabet indicate statistically similar subsets among fish stressed for the last 15 min of the anesthetic exposure by Tukey's multiple range test ($P < 0.05$). Statistical differences between exposed fish and fish exposed and stressed for each treatment by Student's t-test ($P < 0.05$) are indicated by an *. Data represent the mean ± S.E.M. for six fish.


15 min.
30 min.
2 h (recovery)
6 h (recovery)
24 h (recovery)
Chloride (mmol/l)
Control
Stress
Aqui-S
Aqui-S S
Clove Oil
Clove Oil S
Metomidate
Metomidate S
MS-222
MS-222 S
Quin. Sulf
Quin. Sulf S
Quinaldine
Quinaldine S
Salt
Salt S

treatments. Recovery was rapid and complete by 2 h, although, there was a secondary increase at 6 and 24 h after removal of the stress in fish treated with clove oil and quinaldine sulfate.

Plasma glucose concentrations did not increase in control fish after 15 min of low-water stress; however, plasma glucose was significantly higher after stress in all treatment groups except metomidate. Only fish exposed to clove oil and quinaldine sulfate had plasma glucose concentrations higher than control fish stressed without any treatment (Fig. 2). Plasma glucose was somewhat elevated after 2 h of recovery, but there was no difference due to treatment. Glucose concentrations in quinaldine sulfate-treated animals were higher than controls in the 24-h recovery group.

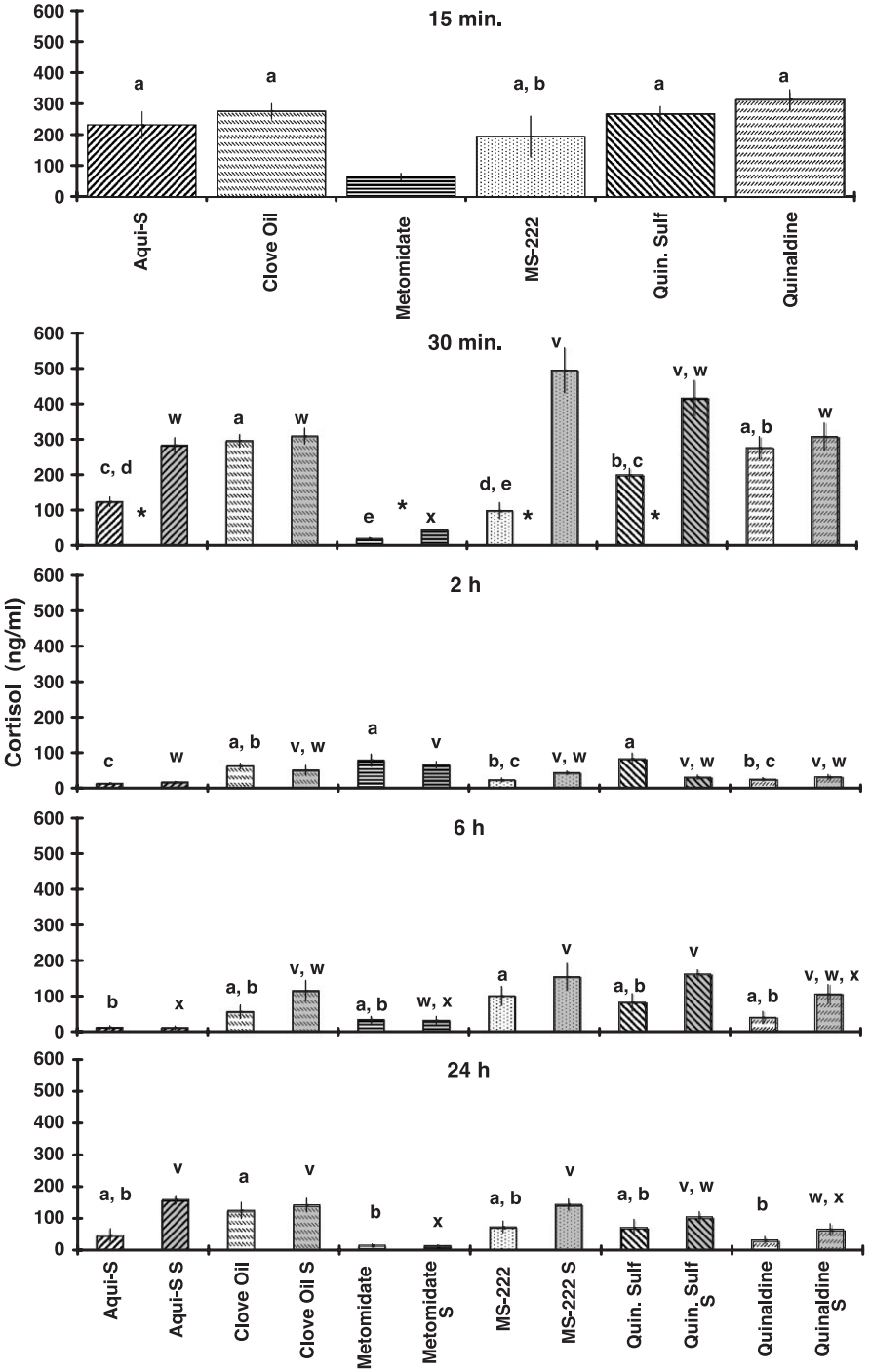
Plasma chloride concentrations in stressed fish were lower than in non-stressed fish only in the control and metomidate-treated fish (Fig. 3). Other plasma chloride changes were not dramatic or consistent. Animals treated and stressed had concentrations similar to those of the controls after 30 min. Stressed control fish and those treated with MS-222 had lower chloride concentrations than all other stressed groups after 2 h of recovery (Fig. 3). After 24 h of recovery, fish stressed in quinaldine had chloride concentrations higher than stressed controls or those stressed in metomidate and MS-222. However, none of the changes in plasma chloride were dramatic.

3.3. Treatment with NaCl combined with anesthetics

Plasma cortisol concentrations of fish were similar after 15 min of exposure in all treatments except metomidate with salt, which was significantly lower than all other treatments except MS-222 (Fig. 4). After 30 min of exposure, cortisol concentrations remained higher than controls in all treatments except metomidate. Some reduction in cortisol concentration was apparent in fish treated with Aqui-S® and MS-222. Recovery to control concentrations was rapid in all groups. After 2 h, metomidate and quinaldine sulfate were higher than all the other treatment groups. A second, slight increase occurred in quinaldine sulfate-treated fish in the 24-h sample. Clove oil- and MS-222-treated animals had the highest cortisol concentrations and metomidate and quinaldine had the lowest at that time (Fig. 4).

Glucose concentrations were significantly lower in fish treated with Aqui-S® for 15 min than fish treated with metomidate or with MS-222 (Fig. 5). Glucose concentrations in all groups after a 30-min exposure were similar. Recovery was similar in all treatment groups. The changes in plasma glucose were quantitatively small both during exposure and recovery.

Fig. 3. Plasma chloride concentrations of sunshine hybrid striped bass exposed to low concentrations of several anesthetics for 30 min (open bars) or for 15 min and then exposed to a low-water stress (S after label, shaded bars) followed by a recovery period of 24 h. Letters from the first part of the alphabet indicate statistically similar subsets among fish exposed to the anesthetics only and letters from the last part of the alphabet indicate statistically similar subsets among fish stressed for the last 15 min of the anesthetic exposure by Tukey's multiple range test ($P < 0.05$). Statistical differences between exposed fish and fish exposed and stressed for each treatment by Student's t-test ($P < 0.05$) are indicated by an *. Data represent the mean ± S.E.M. for six fish.


15 min.
30 min.
2 h
6 h
24 h
Cortisol (ng/ml)
Aqui-S
Aqui-S S
Clove Oil
Clove Oil S
Metomidate
Metomidate S
MS-222
MS-222 S
Quin. Sulf
Quin. Sulf S
Quinaldine
Quinaldine S

Plasma chloride concentrations were significantly lower in fish treated with metomidate and MS-222 after 15 min of exposure. This pattern was observed throughout the experiment (Fig. 6), and continued during recovery.

3.4. Effects of stress during exposure to NaCl and anesthetics

Fifteen minutes of low-water stress increased the plasma cortisol concentration above that in unstressed fish in all groups except clove oil and quinaldine (Fig. 4). Cortisol concentrations in fish exposed to low-water stress were significantly higher in fish exposed to MS-222 and lowest in fish exposed to metomidate. Recovery was rapid and similar in most groups after 2 h. A secondary increase occurred after 6 and 24 h of recovery and was most apparent in fish that received both treatment and stress other than metomidate (Fig. 4).

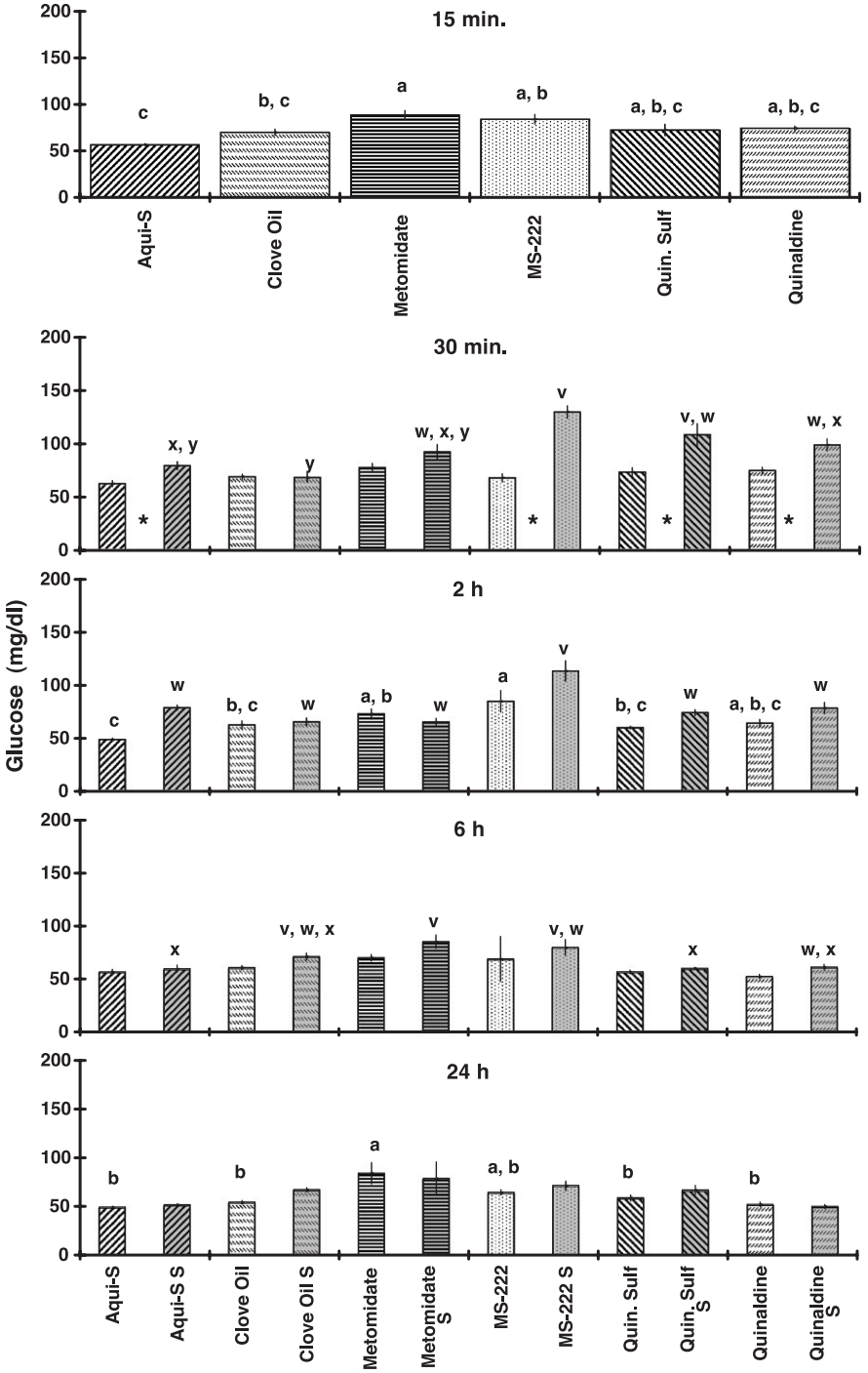
Low-water stress resulted in increased glucose concentrations in all groups except clove oil and metomidate (Fig. 5). Glucose concentrations in stressed fish exposed to MS-222 were significantly higher than those exposed to clove oil (Fig. 5). Other treatments resulted in intermediate glucose concentrations. Glucose concentrations in fish stressed in MS-222 remained higher than most other treatments at 2 h of recovery. Glucose concentrations in all groups were similar by 24 h of recovery.

Stress resulted in higher plasma chloride concentrations in fish exposed to Aqui-S® and lower concentrations in those exposed to quinaldine (Fig. 6). Plasma chloride concentrations in other groups of stressed fish were similar to those fish that were exposed to the anesthetic and not stressed. Fish exposed to metomidate or MS-222 had the lowest plasma chloride of all the treatments and remained so throughout the recovery phase.

4. Discussion

Metomidate was the only compound tested that suppressed the cortisol response. Fish stressed while exposed to metomidate, both alone and in combination with NaCl, had cortisol concentrations slightly, but significantly, higher than exposed non-stressed fish. These cortisol concentrations were much lower than those of any other treatment group of stressed fish. Metomidate [DL-1-(1-phenylethyl)-5-(metoxycarbonyl) imidazole hydrochloride] is the methyl derivative of etomidate. Metomidate and etomidate are non-barbiturate hypnotics which have been shown to reduce plasma cortisol concentrations in mammals (Preziosi and Vacca, 1982; Fraser et al., 1984), and fish, including red drum *Sciaenops*

Fig. 4. Plasma cortisol concentrations of sunshine hybrid striped bass exposed to 5 g/l NaCl combined with low concentrations of several anesthetics for 30 min (open bars) or for 15 min and then exposed to a low-water stress (S after label, shaded bars) followed by a recovery period of 24 h. Letters from the first part of the alphabet indicate statistically similar subsets among fish exposed to the anesthetics only and letters from the last part of the alphabet indicate statistically similar subsets among fish stressed for the last 15 min of the anesthetic exposure by Tukey's multiple range test ($P < 0.05$). Statistical differences between exposed fish and fish exposed and stressed for each treatment by Student's *t*-test ($P < 0.05$) are indicated by an *. Data represent the mean ± S.E.M. for six fish.

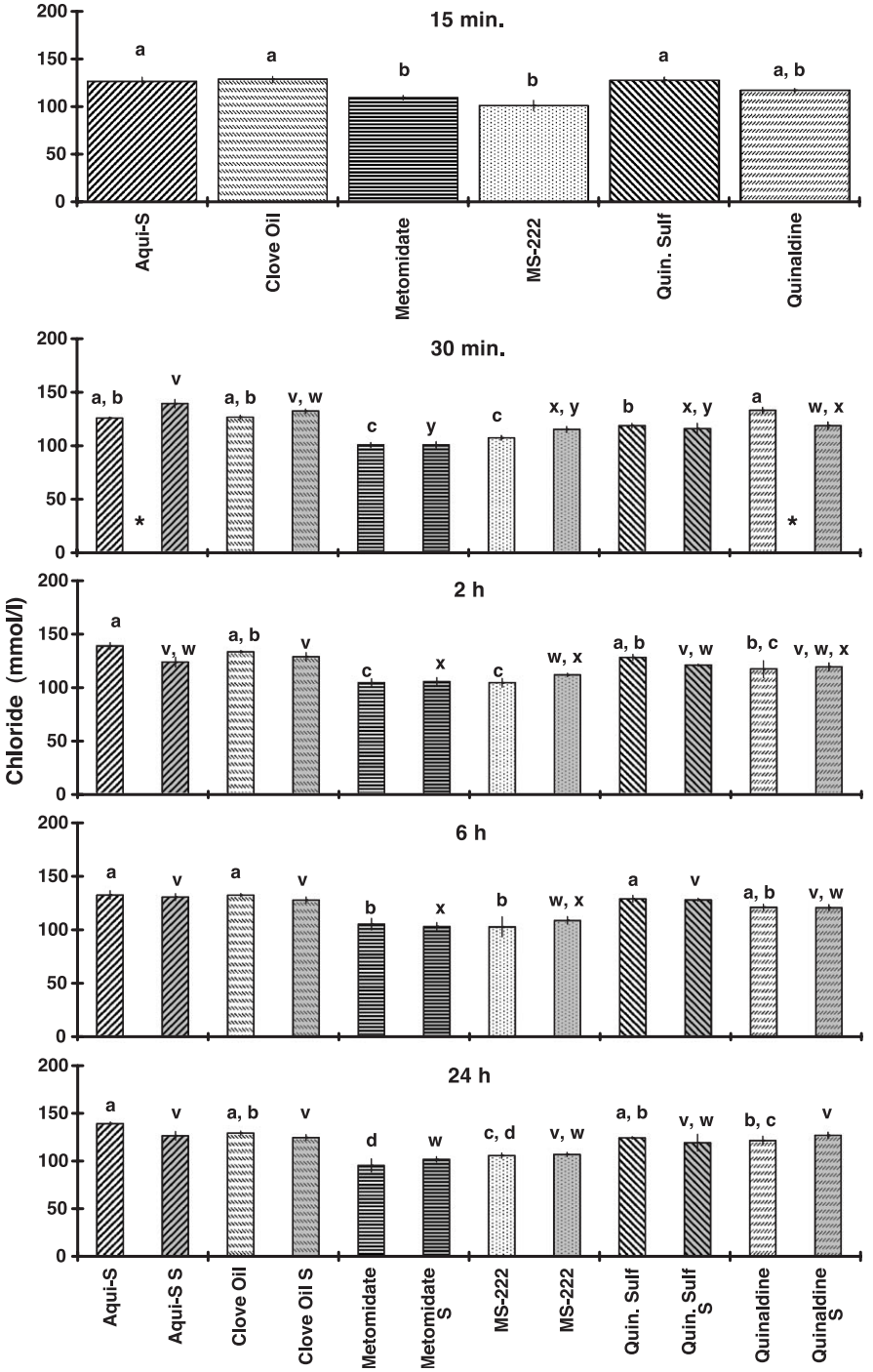

15 min.
30 min.
2 h
6 h
24 h
Glucose (mg/dl)
Aqui-S
Aqui-S S
Clove Oil
Clove Oil S
Metomidate
Metomidate S
MS-222
MS-222 S
Quin. Sulf
Quin. Sulf S
Quinaldine
Quinaldine S

ocellatus (Thomas and Robertson, 1991), Atlantic salmon *Salmo salar* (Olsen et al., 1995) and channel catfish *Ictalurus punctatus* (Small, 2003). Etomidate inhibits the mitochondrial cytochrome P450-dependent enzymes that catalyze the synthesis of cortisol in mammals (Wagner et al., 1984; Vanden Bossche et al., 1984) and metomidate is thought to have the same action.

All of the compounds used here, except metomidate, have been routinely used for some phase of raising fish. The use of NaCl to reduce mortality when fish are handled is a common practice and exposure to NaCl alone induced a more transient cortisol increase than the response to the other compounds. However, NaCl did not reduce the stress response to the anesthetics nor did it reduce the cortisol response to stress in fish exposed to the anesthetics. These data clearly demonstrate that exposure to all these compounds, except metomidate, can elicit a stress response even at low concentrations. Red drum treated with immobilizing doses of MS-222 and quinaldine sulfate had a robust cortisol response to exposure, whereas metomidate completely blocked the response. Metomidate also blocked cortisol stimulation in red drum following injections of ACTH, whereas MS-222 or quinaldine sulfate did not block the response (Thomas and Robertson, 1991). Metomidate at concentrations similar to those used here blocked the increase of cortisol in Atlantic salmon whereas only higher concentrations of clove oil and Aqui-S® were effective in decreasing cortisol secretion (Iversen et al., 2003). Suppression of cortisol with clove oil and Aqui-S® was achieved with anesthetizing concentrations of the anesthetics.

Sea bream *Sparus aurata* and rainbow trout *Onchorynchus mykiss* had increased cortisol concentrations in response to handling while exposed to clove oil (Tort et al., 2002); however, Small (2003) reported no cortisol increase in channel catfish anesthetized with clove oil. Clove oil has been used for sampling tropical reef fish populations (Ackerman and Bellwood, 2002) and in field trials with wild migrating sockeye salmon *Onchorynchus nerka* (Woody et al., 2002). Clove oil is a mixture of 85–95% eugenol with the rest of the active ingredients made up by isoeugenol and methyleugenol. The latter compound is carcinogenic in rodents and raises doubt about the likelihood of clove oil receiving FDA approval. None of these compounds are approved for use in food fish although eugenol is under investigation by the FDA (US FDA, 2002). Aqui-S® is a proprietary fish anesthetic approved as a "food safe" anesthetic in New Zealand, Australia, and is under review by the US FDA. The active ingredient is prepared as a solution of 540 g/l of isoeugenol with other ingredients and is recommended for use in salmonids at 17 mg/l by the manufacturer. Neither of these compounds suppressed the cortisol response of hybrid striped bass. Anesthesia of rainbow trout with Aqui-S® did not diminish the cortisol response (Davidson et al., 2000).

Fig. 5. Plasma glucose concentrations of sunshine hybrid striped bass exposed to 5 g/l NaCl combined with low concentrations of several anesthetics for 30 min (open bars) or for 15 min and then exposed to a low-water stress (S after label, shaded bars) followed by a recovery period of 24 h. Letters from the first part of the alphabet indicate statistically similar subsets among fish exposed to the anesthetics only and letters from the last part of the alphabet indicate statistically similar subsets among fish stressed for the last 15 min of the anesthetic exposure by Tukey's multiple range test ($P < 0.05$). Statistical differences between exposed fish and fish exposed and stressed for each treatment by Student's *t*-test ($P < 0.05$) are indicated by an *. Data represent the mean ± S.E.M. for six fish.


15 min.
30 min.
2 h
6 h
24 h
Chloride (mmol/l)
Aqui-S
Aqui-S S
Clove Oil
Clove Oil S
Metomidate
Metomidate S
MS-222
MS-222
Quin. Sulf
Quin. Sulf S
Quinaldine
Quinaldine S

Most studies of the effects of anesthetics on the stress response have been done at treatment concentrations which induce immobilization, while the concentrations used in the present studies were tested at about 20% of the concentration required to produce Stage 3 anesthesia in 2–5 min. Hauling hybrid striped bass in the same concentration MS-222 as used in the present study resulted in a significant increase in cortisol which was reduced in half by treatment with MS-222 combined with 10 g/l NaCl (Tomasso et al., 1980). We observed no reduction of the cortisol response by combining MS-222 with 5 g/l NaCl. The concentrations of anesthetics used here were selected so that there was no observable change in behavior during a 30-min exposure. This criterion was used because transported fish should be able to maintain locomotor equilibrium to avoid being thrown against the sides and bottom of the container. This level of exposure is similar to the sedation stage (Stage 1) of anesthesia described by Schoettger and Julin (1969), however, we did not measure reaction to external stimuli. We believe the concentration must be close to a dose inducing some degree of sedation since the next highest concentration tested induced some degree of loss of equilibrium (unpublished data).

MS-222 acts as an asphyxiant in fish due to its depressive effects on respiration and central autonomic function (Blahm et al., 1961; Houston et al., 1971; Soivio et al., 1977). The efficacy of many anesthetics in reducing the stress responses to handling has been clearly demonstrated in many teleost fishes (Strange and Schreck, 1978; Tomasso et al., 1980; Davis et al., 1982; Robertson et al., 1988). Immobilizing doses of anesthetics may reduce cortisol stress responses by affecting the perception of the stressor (Schreck, 1981). The concentration used for sedation may not have sufficiently suppressed this perception. Experimental fish thrashed about which indicates an awareness of the low-water stressor.

The plasma cortisol and glucose stress responses to capture and handling have been reported to be reduced by short-term treatment with immobilizing doses of MS-222 in chinook salmon *O. tshawytscha* (Strange and Schreck, 1978) and hybrid striped bass (Tomasso et al., 1980). The low-water stress imposed in this study did not result in an increase of glucose in the controls or in metomidate-treated fish, however, glucose did increase when fish were stressed with the other test compounds alone. The glucose increase in fish stressed after treatment with a combination of NaCl and the anesthetics was lower and was not significant in fish treated with clove oil and metomidate. The plasma cortisol pattern after stress in fish treated with the combination of NaCl and anesthetics was generally lower except in fish exposed to MS-222 and quinaldine sulfate.

Metomidate may prove useful in transporting fish since the cortisol stress response is dramatically suppressed by concentrations that allow the fish to maintain equilibrium. Fish that are transported while immobilized can be damaged by contact with the sides and bottom of the tank.

Fig. 6. Plasma chloride concentrations of sunshine hybrid striped bass exposed to 5 g/l NaCl combined with low concentrations of several anesthetics for 30 min (open bars) or for 15 min and then exposed to a low-water stress (S after label, shaded bars) followed by a recovery period of 24 h. Letters from the first part of the alphabet indicate statistically similar subsets among fish exposed to the anesthetics only and letters from the last part of the alphabet indicate statistically similar subsets among fish stressed for the last 15 min of the anesthetic exposure by Tukey's multiple range test ($P < 0.05$). Statistical differences between exposed fish and fish exposed and stressed for each treatment by Student's *t*-test ($P < 0.05$) are indicated by an *. Data represent the mean ± S.E.M. for six fish.

Only quinaldine induced an increase in plasma glucose after 30 min and the increase resulting from the stress in all cases was transient; recovery in most cases was complete by 2 h. Fish stressed while exposed to metomidate alone or combined with NaCl had no elevation of glucose. Our data are similar to those of Thomas and Robertson (1991) in red drum and Limsuwan et al. (1983) in channel catfish. Glucose increases are due to mobilization of liver glycogen stores caused by sympathetic nervous system activation resulting in catecholamine secretion (Mauzeaud and Mazeaud, 1981). Preventing excessive mobilization of glucose would conserve glycogen stores in the liver.

The lack of a consistent change in plasma chloride may have been because the stress was not severe enough or the recovery phase was not long enough. A decrease in plasma chloride in largemouth bass *Micropterus salmoides* transported in fresh water was not apparent until 12 h and continued for up to 5 days after the 30-h trip (Carmichael et al., 1984).

Inhibition of the cortisol response consistently observed with metomidate is generally thought to prevent the immunosuppressive action of cortisol (Barton et al., 1987; Maule et al., 1989). Confinement stress and oral administration of cortisol have been shown to increase susceptibility to infection by *Ichthyopthirius multifiliis* in channel catfish (Davis et al., 2002, 2003), and stress increases susceptibility to bacterial infection in many species of fish (Ortuno et al., 2001; Walters and Plumb, 1980; Maule et al., 1989; Wise et al., 1993). Thomas and Robertson (1991) have suggested that an adequate corticosteroid response may be essential for resistance to severe physical trauma, and increased post-surgery mortality has been reported in patients anesthetized with metomidate (Ledingham and Watt, 1983). In a preliminary study with hybrid striped bass, fish stressed in untreated water and allowed to recover in metomidate were more lethargic and did not recover as well as control fish. The secretion of cortisol due to stress is a natural part of the stress response and may not be harmful and perhaps even beneficial to the survival of the fish, particularly acute responses. A transient, relatively small, elevation of cortisol may not reduce immunocompetency resulting in increased disease susceptibility. Acute stress in chinook salmon resulted in a depression of antibody-producing cells 4 h after stress, however, disease resistance was enhanced 24 h after the stress (Maule et al., 1989). Further, stress-induced enhancement of immune function has been shown in mammalian systems exposed to moderate stress (Dhabhar and McEwen, 2001). The increase of cortisol due to transportation stress (Carmichael et al., 1984) and exposure to MS-222 (Thomas and Robertson, 1991) was found to be proportional to the degree of stress. Prior exposure to MS-222, quinaldine sulfate and metomidate were equally effective in preventing cortisol and glucose stress responses to a 2-min handling stressor in red drum (Thomas and Robertson, 1991). Only metomidate was effective in reducing the plasma cortisol and glucose stress effect when hybrid striped bass were exposed for 30 min with or without an additional stressor. Metomidate used in low concentrations may prove useful in reducing cortisol and hyperglycemia often associated with handling and transportation stress in fish, however, more studies that examine survival with metomidate-treated fish need to be done. Metomidate has been reported not to have analgesic (relief from pain) properties (Horsberg and Samuelsen, 1999 as reported by Iversen et al., 2003), and would be inappropriate for use under conditions which inflict pain. Metomidate should be a useful compound to help understand which of the stress components are mediated by

cortisol. Any of these compounds found to be useful must be cleared by FDA, or its equivalent in countries other than the USA, for use in food fish.

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